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Label-free and real-time detection of specific binding of IgG proteins by oblique-incidence reflectivity difference method

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The entire interaction process between anti-mouse immunoglobulin-G (IgG) and mouse IgG with a concentration range from 1 mg/mL to 0.25 mg/mL has been label-free detected by the oblique incidence reflectivity difference (OIRD) method. We achieved the two-dimensional scanning images of OIRD $\mathrm{Re}\{\Delta_p - \Delta_s\}$ intensities before and after interactions as well as the curves of reaction dynamic processes. The experimental results suggest that OIRD method not only can label-free distinguish the different concentrations of proteins and detect biological microarrays, but also can real-time monitor the kinetic processes of biomolecular interactions.

oblique incidence reflectivity difference (OIRD), label-free and real-time detection, biomolecular interactions

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It is still a big challenge to obtain a label-free and high throughput detection method for biological microarrays, because up to now, the label-based detection methods, such as radioactive labeling, chemiluminescence, and fluorescence, are widely applied in the monitoring of biomolecular interactions for their high sensitivity and high throughput [1,2]. However, the whole procedures including labeling and detecting are very costly and time-consuming. Especially, the extrinsic label probably impacts the biomolecular characteristics and the natural activities [3,4]. So far, though the surface plasmon resonance (SPR) is the most widely used method that has been applied successfully to the detection of label-free biological microarrays [5,6], SPR has not been considered to be a high-throughput method yet. In addition, label-free and real-time detection of dynamic processes of biomolecular interactions can obtain the quantitative information of interaction kinetics; therefore, label-free and real-time detection is more attractive for biological investigation. Oblique-incidence reflectivity difference (OIRD) method as a high sensitive and label-free technique has been used in the detection of biological microarrays [7]. We have reported the label-free detection of biomolecular microarrays and real-time detection of biomolecular interactions using OIRD method [8–14]. In this work, we report on the label-free and real-time detection of interaction processes of IgG proteins by OIRD method. The experimental results suggest that the OIRD method can measure the entire interaction process of biological molecules and has potential application for the label-free detection of biological microarrays.

1 Experimental

Figure 1 shows the schematic diagram of OIRD setup for the label-free and real-time detection of IgG proteins. As mentioned in our previous work [11], the probe beam is a p-polarized He-Ne laser with 632.8 nm wavelength, and first passes through a photoelastic modulator (PEM90) which induces the probe beam to oscillate between p- and

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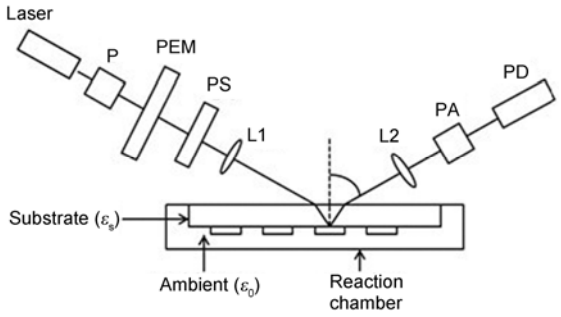

Figure 1 The schematic diagram of OIRD setup for the label-free and real-time detection of IgG protein microarray. Laser, a 7 mW He-Ne laser; P, polarizer; PEM, photoelastic modulator; PS, 1/2 plate; L1 and L2, lens; PA, polarization analyzer; PD, photodiode detector.

s-polarization with the modulated frequency Ω=50 kHz. Then the modulated polarized light passes through a phase shifter (PS), which causes a variable phase between p- and s-polarization components, and is focused on the surface of protein microarray at an incident angle of 60°. The protein microarray is covered on the chamber and the sample surface is on contact with the liquid phase. The incident light is reflected by the sample surface, and the intensity of resultant beam is detected by a silicon photodiode (PD) and two lock-in amplifiers. At oblique incidence, for the different surface, the surface reflectivities of p- and s-polarized light are different as the different of the surface layer thickness and/or the complex dielectric constant. Let us define r_{p0} and r_{s0} as the reflectivities from the bare microarray surface without protein molecules for p- and s-polarized light, and r_p and r_s be the reflectivities from the protein covered surface, respectively. The changes of p- and s-components in reflectivity are defined as $\Delta_p=|(r_p-r_{p0})/r_{p0}|$ and $\Delta_s=|(r_s-r_{s0})/r_{s0}|$, respectively. The difference in reflectivity change is $\Delta_p-\Delta_s$. In the experiment, we measure the changes of the OIRD real parts $\mathrm{Re}\{\Delta_p-\Delta_s\}$.

In this study, we selected the polymer brush functionalized glass slide [15] as the microarry substrates, mouse immunoglobulin-G (IgG) as the target and goat anti-mouse IgG as the probe (all the IgG was purchased from KPL Inc, USA). We used conventional procedure [16] to fabricate the IgG microarrys. Firstly, the mouse IgG with different concentrations, 1 mg/mL, 0.5mg/mL and 0.25mg/mL, were printed at the surface of polymer brush functionalized slide using a spotting robot microsystem (Personal ArrayerTM 16, CapitalBio Corporation CA). As shown in Figure 2, every two columns is printed with IgG concentration decreasing from top to bottom, and each row contains five same spots. The center to center separation of the adjacent IgG spots is about 300 μm, and the average diameter of the sample spots is about 100 μm. Secondly, the mouse IgG microarry was washed with 1×PBS (phosphate buffered saline) for 5 min and $\mathrm{ddH_2O}$ for 5 min in order to remove the redundant IgG and salt precipitate, and then the microarry was dried using the nitrogen. Thirdly, the IgG microarry was mounted on the reaction chamber as shown in Figure 1.

We measured the entirely interaction processes of the IgG proteins. Before interaction, we filled the reaction chamber with 1×PBS, and label-free measured the two dimensional scanning image of the mouse IgG microarray with different concentrations. Then, 1×PBS was substituted with 0.01 mg/mL goat anti-mouse IgG in the reaction chamber, the detection light spot moved back and forth along the centerline of the six sample spots in left a column of the microarray, and we real-time monitored the interaction dynamic processes of the six sample spots. When the interaction finished, we again measured the two dimensional scanning image of the IgG microarray.

2 Results and discussion

Figure 2(a) and (b) display the two dimensional scanning images of the OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ intensities measured before and after reaction, respectively. From Figure 2(a) and (b), one can clearly see that the gray values of sample spots in different concentrations are dropped with the concentration decreasing, indicating that it is more advanced than that of label methods, and OIRD method can label-free distinguish the different concentrations of proteins. Figure 2(c) is the differential image obtained by subtracting Figure 2(a) from Figure 2(b), showing the specificity binding between mouse IgG with different concentrations and anti-mouse IgG.

Figure 3 shows the variations of the OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ signal with time, which corresponded to the reaction dynamic processes between anti-mouse IgG and mouse IgG with different concentrations. The curves A, B, and C correspond to the different concentration IgGs, 1mg/mL, 0.5mg/mL and 0.25mg/mL, respectively, and the same concentration has two sample spots. The data in curve D are obtained from the slide surface without mouse IgG close to zero, meaning that anti-mouse IgG do not interact with the slide. From the curves A, B, and C, we can see that the interaction process is an exponential behavior; the IgG concentration higher, the $\mathrm{Re}\{\Delta_p-\Delta_s\}$ signal intensity bigger. The curves A, B, and C tend to saturate and reach an

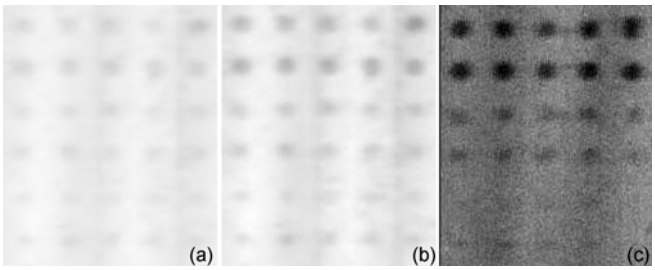

Figure 2 The two dimensional scanning images of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ intensity signals. (a) Before reaction; (b) after reaction; (c) the differential image of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ signal obtained by subtracting Figure 2(a) from Figure 2(b).

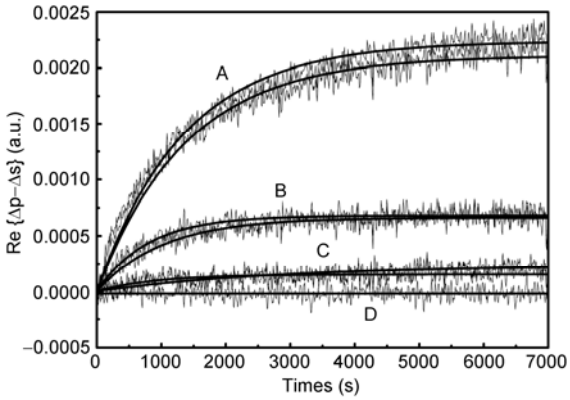

Figure 3 The variations of OIRD Re{Δ_p–Δ_s} signal with time. Curves A, B, C and D correspond to different concentrations of IgG, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL and 0 mg/mL (from the slide surface without mouse IgG), respectively.

equilibrium state with the time increasing, meaning the specific binding finished.

3 Conclusion

We measured the entire interaction process between anti-mouse IgG and mouse IgG with different concentrations, including label-free detections before and after interactions as well as label-free monitor of the interaction kinetic processes by the OIRD method, and achieved the two dimensional scanning images of the OIRD Re{Δ_p–Δ_s} intensities before and after interactions, and the curves of reaction dynamic processes. The experimental results suggest that OIRD method not only can label-free distinguish the different concentrations of proteins and detect the biological microarrays, but also can real-time monitor the kinetic processes of biomolecular interactions. Further study on the kinetic process of biomolecular interactions and the quantitative analysis of kinetic processes is planned.

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